

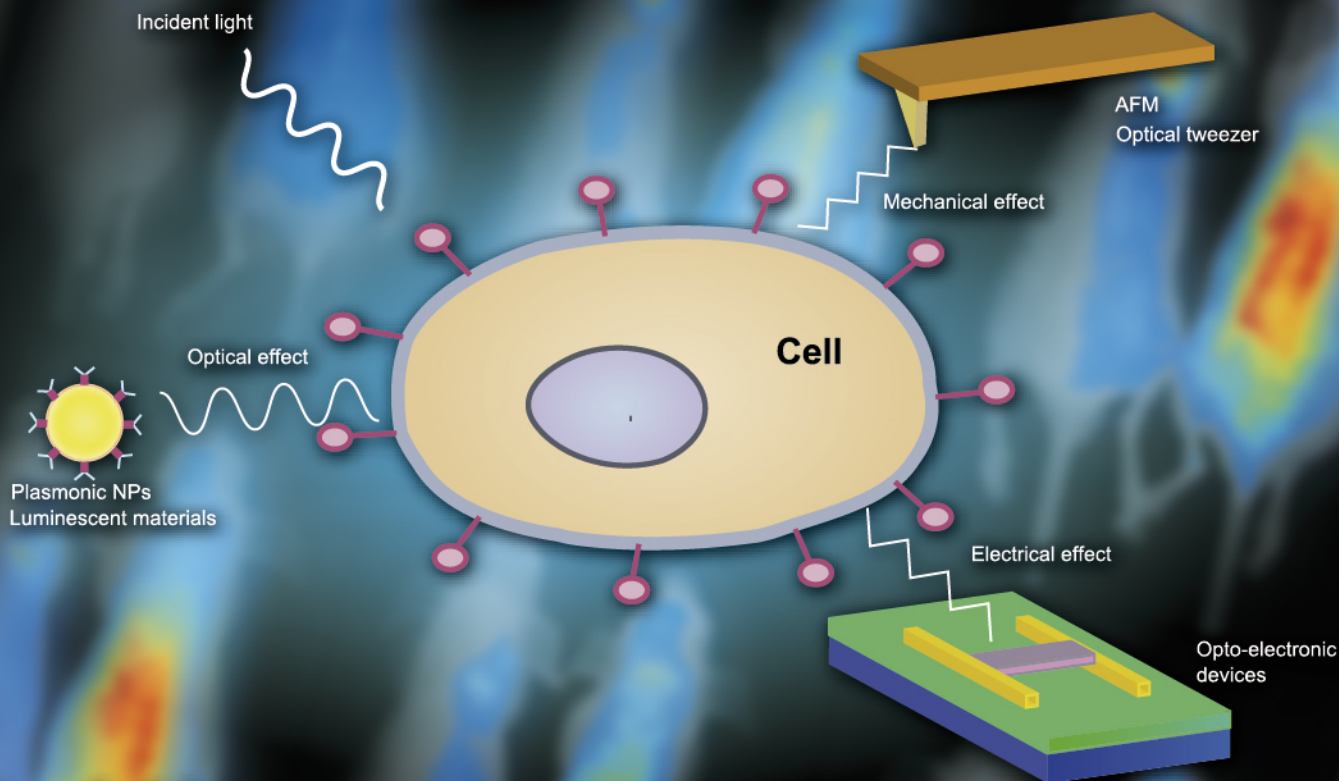
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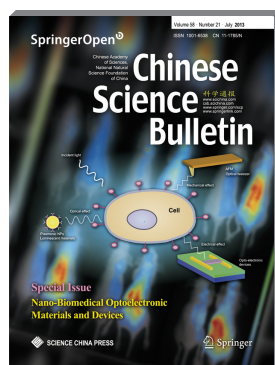
Special Issue

Nano-Biomedical Optoelectronic  
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**COVER** Recently, nanotechnology for biomedical research has received extensive attention, and considerable progress has been made in this area. Nanotechnology encompasses many advanced research areas such as nanocharacterization and nanomeasurement based on optoelectronic technology, nanomaterials with optically modulated parameters, nanophotonics and nano-optoelectronics. These research areas cover important applications such as detection and manipulation of bionanostructures, *in vitro* and *in vivo* characterization of biomolecules for clinical diagnosis, imaging and detection of single cells, cell masses and tissues, functional nanomaterials for dietetic therapy, and nanoscale medical transportation systems. As a result, nano-optoelectronics for biomedical research has developed into an important and highly promising interdisciplinary field. The background photograph on the cover shows *in vivo* non-invasive NIR fluorescence images of nano-drug carriers. Targeting molecules favors the *in vivo* distribution of drug molecules in the target region, which improves therapeutic efficiency (see the special issue: Nano-Biomedical Optoelectronic Materials and Devices).

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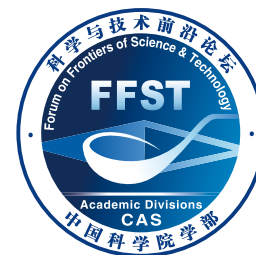
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## Generalized multiparticle Mie modeling of light scattering by cells

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The development of nanobiology requires a fundamental understanding of the interaction features between light and cells as well as cells containing nanoparticles. In this study, the generalized multiparticle Mie (GMM) theory was employed to calculate the scattering properties of cells under refractive index matching conditions. The angular distribution of scattered light is statistically averaged to obtain a good fit for the experimental results. Based on a simplified cell model, the variabilities between the scattered light pattern of normal cells and that of cancerous cells were examined. The results indicate that the small angle scattering is sensitive to the organelle distribution, which could be applied in the diagnostics of cancerous cells. Finally, the effects of cellular uptake of nanoparticles on the scattering pattern was also investigated.

**light scattering, single cell detection, GMM theory, cellular uptake**

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Optical diagnostic techniques have grown rapidly in recent years which provide the ability to characterize the tissue structure and cell morphology at cellular and sub-cellular level [1–4]. Many of these techniques, such as optical tomography, flow cytometry and elastic scattering spectroscopy, rely on correlating the scattering properties of tissue to infer its physiological state. The spatial distribution of the scattered light intensity depends on cell's morphology and the polarization states of incident light [5,6]. While the complete solution to the inverse problem of light scattering from single cells still remains a challenge, we can extract cellular morphological information from the scattered light in specific angular ranges or the overall pattern to discriminate different cell types.

Theoretical calculations can provide a better understanding of light interaction features with biological cells, and give rise to the developments of non-invasive, label-free optical diagnostic methods [7,8]. Biological cells can be consid-

ered as compound dielectric objects consisting of organelles with different refractive indices. Electromagnetic approaches should be used rather than geometrical optics based techniques, because the sizes of the scatters are small or comparable to the wavelength.

For decades, light scattering from single cells has been investigated in many scientific and research literatures [9–13]. Dunn et al. [9] employed the finite-difference time-domain (FDTD) method to simulate light scattering from a single uniform cell for the first time. Tanev et al. [7] reported the application of FDTD method to the model of light scattering from cells for application in advanced cell imaging based on optical phase contrast microscopy (OPCM) techniques. Su et al. [13] suggested that the mitochondria inside the cell change the small angle forward scatter intensity distributions as in the FDTD simulations. The FDTD is a three dimensional full wave method which can characterize arbitrarily inhomogeneous dielectric objects. However, these methods have some limitations. First, the numerical methods require very high consumption of computational resources. For in-

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stance, it would cost over 12 GB of memory and 24 CPU h to simulate a yeast cell (diameter, 2  $\mu\text{m}$ ) by using the FDTD method [14]. Second, most simulations for cell scattering were carried out in one direction. In realistic conditions, the instruments have insufficient sensitivity to detect individual cells. Thus, it is inevitable to accumulate and statistically average the light scattering from a group of cells with different orientations.

The purpose of this paper is twofold: first, to present the flexibility of the GMM theory for calculating scattered light pattern of single cells; and second, to investigate the effects of cell uptake of nanoparticles on the scattering pattern. The GMM theory is an extension of Mie theory to the multi-particle case, which require much lower computational resources compare to the numerical methods. Based on reasonable approximations, a simplified GMM model was set up for normal and cancerous cells. To the best of our knowledge, it is the first time that the GMM theory has been applied in the light scattering simulations for single cells.

## 1 Methods

### 1.1 GMM method

The GMM theory is a semi-analytical solution to light scattering by an arbitrary ensemble of spheres [15]. The incident and scattered fields are expanded in vector spherical wave functions around each sphere in the GMM theory. By applying the boundary conditions at the surface of each particle associated with vector translation theorems, we can yield the following large-scale system of linear equations:

$$\begin{aligned} a_{mn}^l &= a_n^{-l} p_{mn}^{-l} - a_n^{-l} \sum_{j \neq l}^{(1,L)} \sum_{\nu=1}^{N_j} \sum_{\mu=-\nu}^{\nu} (A_{mn\mu\nu}^{jl} a_{\mu\nu}^j + B_{mn\mu\nu}^{jl} b_{\mu\nu}^j), \\ b_{mn}^l &= b_n^{-l} q_{mn}^{-l} - b_n^{-l} \sum_{j \neq l}^{(1,L)} \sum_{\nu=1}^{N_j} \sum_{\mu=-\nu}^{\nu} (B_{mn\mu\nu}^{jl} a_{\mu\nu}^j + A_{mn\mu\nu}^{jl} b_{\mu\nu}^j). \end{aligned} \quad (1)$$

The scattered fields are determined by the partial scattered field expansion coefficients ( $a_{mn}$ ,  $b_{mn}$ ), which can be obtained by solving the equations numerically.

The linear relationship between the incident and the scattered far-field components, that are parallel and perpendicular to the scattering plane defined by the direction of propagation of the plane incident wave and the scattering direction, can be expressed concisely in matrix form:

$$\begin{pmatrix} E_{\parallel s} \\ E_{\perp s} \end{pmatrix} = \frac{e^{ik(r-z)}}{-ikr} \begin{pmatrix} S_2 & S_3 \\ S_4 & S_1 \end{pmatrix} \begin{pmatrix} E_{\parallel i} \\ E_{\perp i} \end{pmatrix}, \quad (2)$$

where  $S_i$  ( $i = 1, 2, 3, 4$ ) are the four elements of the amplitude scattering matrix, which can be rigorously solved in terms of the partial scattering coefficients and the geometry of the scatter. Detailed descriptions of the algorithm can be found elsewhere [16,17].

### 1.2 Cell model and parameters

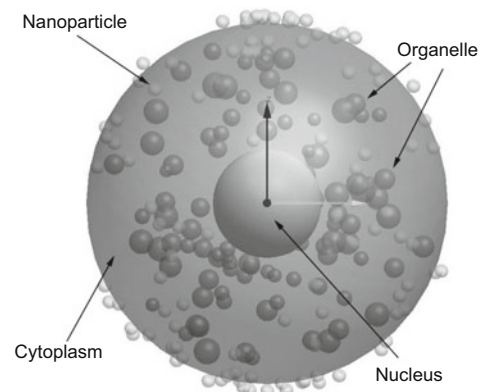
GMM theory can only calculate aggregate of non-intersecting spheres. To simulate light scattering from cells by GMM theory, we need to make some approximations. First, the background is matched to that of cytoplasm for more accurate modelling of scattering by cells embedded in tissue. Second, the nucleus and organelles within the cell are assumed to be spherical. Finally, it does not take into account the effects of membrane on scattering, because the GMM method is unable to resolve the cell membrane. This is a reasonable approximation, previous study has found that the membrane has a relatively small contribution to scattering [5].

Based on the above assumptions, the cell can be simply modelled as a dielectric object consisting of any number of organelles varying with sizes and reflective indices as illustrated in Figure 1. The cell has a major diameter of 10  $\mu\text{m}$ , and the diameter of the nucleus is 4  $\mu\text{m}$ . The randomized organelles and nano particles are generated by a Monte Carlo script which can produce different distributions. The refractive indices of cytoplasm and nucleus are assumed to be 1.36 and 1.38, respectively, and the refractive indices of organelles uniformly distributed in the range of  $1.40 \pm 0.05$  [6]. The dielectric functions for gold and  $\text{Fe}_3\text{O}_4$  are taken from the tabulated data measured by Johnson et al. [18] and Schlegel et al. [19], respectively.

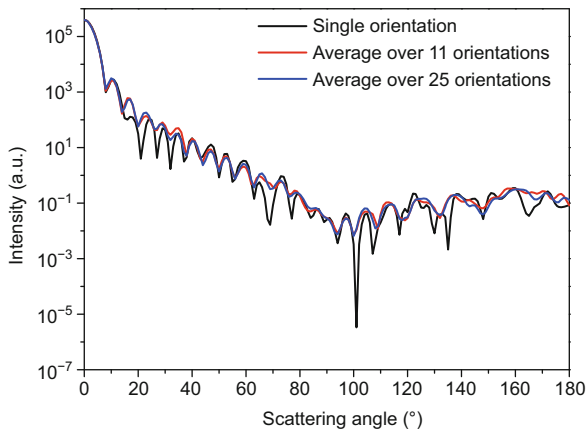
The calculations were performed on a normal PC (Intel i7-2600 3.4 GHz) using the modified GMM program developed by Xu [15]. The incident light travels along the  $z$  direction with a wavelength of 500 nm. The expansion order of GMM was set to 50, whose validity had been verified by the calculations in higher order. For a cell containing 500 organelles, the program would require about 150 MB of memory and 20 min of CPU time.

## 2 Results and discussion

Consider a cell with 6.5% organelle density (500 organelles with radii from 200 to 400 nm). Figure 2 shows the perpendicular component of the angular scattered intensity for



**Figure 1** Schematic representation of the cell model used in the GMM calculation.

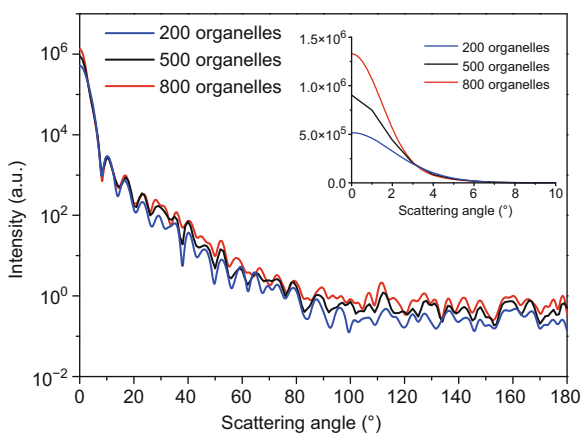


**Figure 2** Perpendicular component of the scattered intensity as a function of scattering angles for the cells with different orientations.

the cells with different orientations. The scattering is highly peaked in the forward direction, so that the scattering intensities are plotted on a logarithmic scale in order to examine the behaviour in large scattering angles. Light with different polarization and direction has different scattered light patterns due to the asymmetry of the cell structure. For the scattering pattern of individual orientation (black curve), the scattered light forms a distinctive interference fringe pattern. For averaged scattering patterns, the intensity variations of interference fringes are greatly reduced. In actual measurements, such as flow cytometry and light scattering spectroscopy, the experimental results are obtained from a large number of cells or cell suspension. It is more suitable to average the scattering intensity. The differences between the two averaged patterns (red and blue curves) are subtle. In the following part, we just plotted the unpolarized scattering patterns which are averaged over 11 orientations.

### 2.1 Effects of organelle quantity and distribution

Figure 3 plots the scattering patterns of cells with different numbers of organelles: 200, 500, 800. The results show that the variation of organelle quantity changes the angular distribution of light scattered intensity. It is clear that the increase



**Figure 3** Angular light scattering distribution of cells with different numbers of organelles.

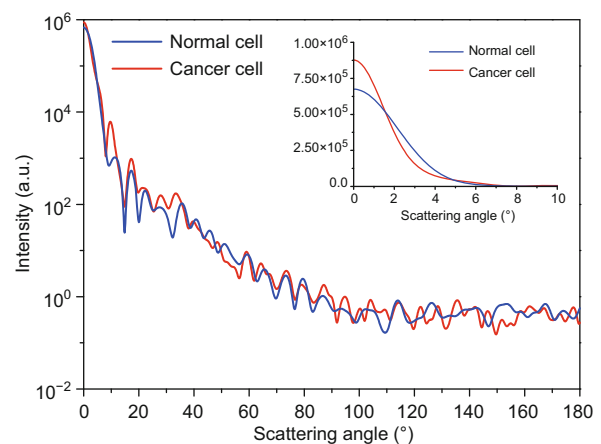
in organelle number enhance the forward scattering intensity at large angles ( $\theta > 30^\circ$ ). For the cell with more organelles, the scattering patterns appear as a smooth curve due to the multiple scattering from large numbers of scatterers. When the small angle intensity ( $0^\circ$ – $10^\circ$ ) is plotted in linear scale, as shown in the inset, an apparent linear increase in forward scattering is also observed.

Figure 4 shows the cases for two type of cells with different organelle distributions which are referred as “normal” and “cancer” cells. For normal cells, the organelles are located on the nuclear periphery; for cancer cells, the organelles are mainly located close to the cell surface [13]. As shown in the inset, the differences in the intensity are evident at small angles. Cancerous cells have a higher intensity than the normal cells at forward angle ( $\theta = 0^\circ$ ). However, the exponential decay coefficient of intensity for cancerous cells is larger than that of the normal cell. Thus, at angles range from  $2^\circ$  to  $5^\circ$ , normal cells have higher intensities.

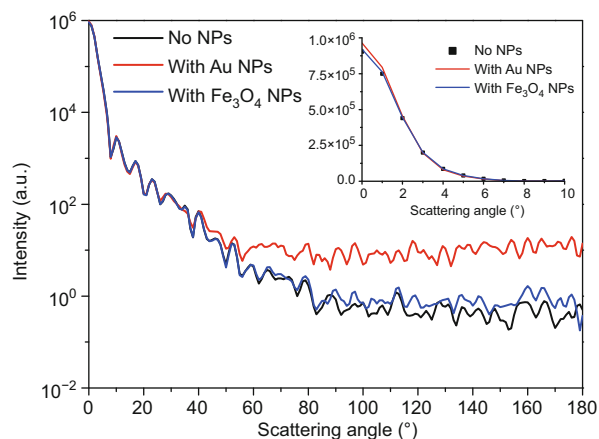
The above results show that the increase in organelle quantity enhances the scattering pattern over all angles, and change in organelle distribution affects the small angle scattering. Our study is consistent with earlier findings studied by FDTD methods [5]. The effect of the organelle quantity and distribution on the light distribution scattering pattern is an example to a cellular based optical diagnosis method. It is anticipated that this feature of small angle scattering could serve as an indicator in the diagnostics of cancerous cells.

### 2.2 Effects of nanoparticle uptake

The results shown in Figure 5 demonstrate the effect of the nanoparticle uptake on the scattered light. There are 500 nanoparticles randomly located in the cell cytoplasm. For contrast, two types of nanoparticles, gold and  $\text{Fe}_3\text{O}_4$  (diameter, 50 nm), are considered in the calculation. It is found that the gold nanoparticles significantly enhance the scattered light intensity at large angles. Specifically, there is an over tenfold increase in side scattering ( $\theta > 90^\circ$ ) compared with



**Figure 4** Comparison of the light scattering distribution of a normal cell with a cancerous cell.



**Figure 5** Angular light scattering distribution of cells in the presence of nanoparticle uptake.

control. However, the  $\text{Fe}_3\text{O}_4$  nanoparticles make little difference on both forward and side scattering. As is known, gold nanoparticles exhibit the ability to resonantly scatter light due to the excitation of surface plasmon resonances. The high scattering cross sections of gold nanoparticles are essential for scattering imaging leading to the enhancement of scattering.

Bohmer et al. [20] conjugated the immunoglobulin antibodies to 40 nm gold particles for labelling lymphocytes. As analysed by flow cytometry, the side scattered signal was enhanced more than tenfold by the gold label. This is in quantitative agreement with our simulation results.

It should be pointed out that the nanoparticles are randomly distributed in the cell cytoplasm in the calculation. However, the cellular uptake of the nanoparticles is very complicated. Further theoretical and experimental investigations are needed to accurately model the spatial distribution of nanoparticles.

### 3 Conclusions

In summary, a semi-analytic method, GMM theory is proposed to calculate the scattering properties of single cells. The GMM theory can greatly reduce the computational resource requirements, which can permit us to perform the calculation on a normal PC. The effects of the organelle distribution and cellular uptake of nanoparticles on light scattering from single cells have been studied. To get a better fit for the realistic situation, the scattering intensities are statistically averaged over different orientations.

The results show that changes in organelle distribution can cause significant changes in the forward light scattering, which could be used to distinguish normal and cancerous cells. The presence of gold nanoparticles greatly increases the side scattering due to the high scattering cross sections

of the gold. Meanwhile, the  $\text{Fe}_3\text{O}_4$  nanoparticles have little effect on the scattered light. We hope this study can give rise to new techniques for classification and quantification of nanoparticle uptake by cells.

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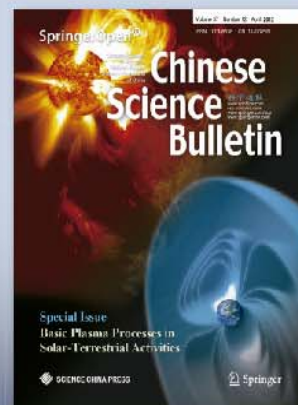
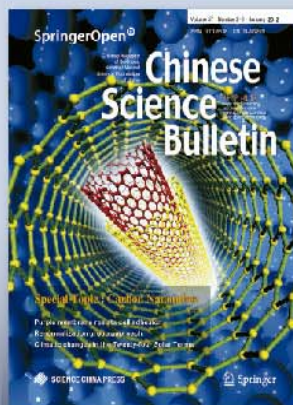


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